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Interactions between Viruses and Bacteria in Patients with Chronic Bronchitis

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The possibility that viral infections of the respiratory tract might predispose to bacterial colonization or infection was studied in 120 patients with chronic obstructive pulmonary disease and 30 control subjects; these individuals were observed for seven years. The ratio of the number of observed to the number of expected associations between viruses and bacteria was 2.43 ($P = 0.037$) for the pair influenza virus and *Streptococcus pneumoniae* and was 2.06 ($P = 0.056$) for influenza virus and *Haemophilus influenzae*. Consistently positive, but not significant, associations were detected between rhinovirus and herpes simplex virus infections and isolations of *S. pneumoniae* and *H. influenzae*. In contrast, isolations of the nonpathogenic *Haemophilus parainfluenzae* could not be related to prior viral infections. Significant rises in titer of antibody to *H. influenzae* were detected on 76 occasions, and 20 (26%) of these antibody rises were associated with viral or mycoplasmal infections during the preceding 120 days. The expected number of such associations was 8.34 (ratio of number observed to number expected, 2.40; $P = 0.08$). These results suggest that viral infections of the respiratory tract in patients with chronic obstructive pulmonary disease are associated with increased colonization by potentially pathogenic bacteria and may also predispose to infection with *H. influenzae*.

The concept that viral infections of the respiratory tract may impair host defenses in a manner that would lead to increased colonization or infection with pathogenic bacteria has been supported by numerous laboratory and clinical studies [1–7]. Patients with chronic obstructive pulmonary disease (COPD) characteristically suffer from recurrent acute and chronic infections due to viruses and bacteria [8, 9], and it has been postulated that interactions between viruses and bacteria may be important in the pathogenesis of this disease [10–12]. Nevertheless, the subject of viral-bacterial interactions in this population of patients has received little investigative attention. Occasional associations between viral and bacterial infections in patients with chronic bronchitis were noted by Fisher et al. [13] and by Lambert and Stern [14]; however, both studies were too limited in scope to allow assessment of the importance of viral-bacterial interactions in this population of patients.

In 1968, we initiated a seven-year study of the role of infection in the pathogenesis of COPD. One hundred twenty patients with COPD and thirty control patients were monitored at bimonthly intervals for evidence of acute respiratory illness and infection with bacteria, viruses, and mycoplasmas. For this report we have analyzed the results of 273 viral infections and of >4,000 bacterial cultures to assess the possibility that viral infections might predispose to bacterial colonization and/or infection in patients with COPD.

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Materials and Methods

One hundred twenty patients with COPD and thirty healthy control subjects were monitored during the seven-year period of 1968–1974 for

evidence of bacterial, viral, and mycoplasmal infections. The diagnosis of COPD was made on the basis of a ratio of forced expiratory volume/sec to forced expiratory vital capacity (FEV_1/FVC) of <69% and a history compatible with this symptom complex.

Each patient was seen in the clinic on a bimonthly schedule; during these visits he or she was questioned regarding the occurrence of acute respiratory illness, immunizations, drug therapy, and disease status. During each clinic visit serum was obtained for antibody studies; throat swabs, nasal secretions, and sputum samples were collected for isolation of viruses and bacteria. In addition, study subjects were instructed to call the investigators at the first sign of acute respiratory illness, and this method of surveillance was amplified by a weekly telephone call to each subject. When acute respiratory illness occurred, a nurse visited the home to obtain samples of respiratory tract secretions for culture.

Throat swabs were placed in Stuarts' transport medium for bacterial culture or in viral transport medium, which consisted of veal infusion broth (Difco, Detroit, Mich.) containing 1% bovine serum albumin, penicillin (1,000 units/ml), and streptomycin (100 μg/ml). Secretions present in the upper nasopharynx were collected for viral culture by washing of each nostril with 5 ml of 0.85% NaCl and mixing of the wash with an equal volume of virus transport medium. When available, sputum samples were also obtained for bacterial and viral culture. Sputum samples and specimens for viral culture were kept at 4 C until inoculation onto appropriate media or tissue culture cells. Inoculation of specimens was generally completed within 6 hr of collection.

Throat swabs and sputum samples were inoculated onto both Columbia agar containing 5% sheep's blood and peptic digest agar for isolation of bacteria. All plates were incubated in a candle jar at 37 C. *Streptococcus pneumoniae* was identified by demonstration of sensitivity to optochin. In the most recent 12 months, gentamicin (5 μg/ml) was added to the Columbia agar to provide more favorable conditions for the isolation of pneumococci [15]. *Haemophilus influenzae* and *Haemophilus parainfluenzae* were isolated from the peptic digest agar plates and were identified by their requirement for X and/or V factors according to the method of Parker and Hoeprich [16].

Sputum, throat, and nasal wash specimens were inoculated onto human embryonic lung (WI-38) cell cultures for isolation of rhinoviruses and herpesviruses. Cell cultures were incubated at 33 C on a roller tube apparatus and were examined daily for evidence of viral CPE. Isolates from cultures exhibiting typical CPE were further characterized by tests for lability to acid and sensitivity to chloroform [17].

Tests for the presence of CF antibody to respiratory viruses, *Mycoplasma pneumoniae*, and *H. influenzae* were performed by the microtiter procedure [18]. Adenovirus antigen; parainfluenza types 1, 2, and 3 antigens; and guinea pig complement were obtained from Flow Laboratories, Inglewood, Calif. Soluble influenza virus types A and B, respiratory syncytial virus, and *M. pneumoniae* CF antigens were obtained from Microbiological Associates, Bethesda, Md. The CF antigen was prepared from *H. influenzae* ATCC strain 19418 (American Type Culture Collection, Rockville, Md.) with use of a modification of the technique of Tunevall [19]. Coronavirus 229E CF antigen was prepared from infected WI-38 cells by the technique of Kapikian et al. [20]. HAI tests for the presence of antibody to coronavirus OC 43 were performed according to the technique of Kaye et al. [21]. Dr. Kenneth McIntosh (University of Colorado Medical Center, Denver, Colo.) kindly supplied seed virus for the preparation of both coronavirus 229E and OC 43 antigen pools.

Tests for CF and HAI antibodies were performed on 2,514 samples of sera collected from 150 patients during the seven-year period of study. A fourfold or greater rise from a previous antibody titer was considered to be evidence of infection with the test agent. Because of the known cross-reactivity between parainfluenza types 1, 2, and 3, the antigen that demonstrated the highest rise in antibody titer was designated as the infecting agent.

Statistical methods. The statistical analysis of viral-bacterial interactions was complicated by the fact that multiple viral and bacterial infections occurred in study patients during a short period. For this reason, there was no simple way

to select a single study group (subjects with viral infection) and a control group (those free of viral infection). An additional problem in this analysis was the considerable variability in the incidence of specific viral infection (e.g., influenza) with the season of the year and from year to year.

The situation was handled by an extension of the Mantel-Haenszel [22] procedure suggested by Mantel and Byar for the analysis of survival after heart transplant surgery [23]. Our adaptation of the Mantel-Byar analysis permitted us to use each study patient on multiple occasions as either a virus-positive or control (virus-negative) individual and to allow for seasonal variability in the incidence of viral infections.

The prospective approach was taken, i.e., patients with, or free of, a viral infection were checked for concurrent or subsequent bacterial infection during the following seven, 30, or 60 days. Below we describe the analysis for concurrent (within seven days) infection. To control for seasonal factors, we constructed a 2×2 contingency table for each day on which one or more patients presented with viral infection (table 1). Control patients, indicated in table 1 as virus-negative, were selected from those who were free of virus. The requirement that control observations be made on the same day as observation of a case was too restrictive. Therefore, controls were allocated to the table containing the closest date of viral infection, but no control subject whose observation was >60 days from that of a case was included.

Table 1. Schematic cross-tabulation of numbers of patients with viral infections on any given day vs. whether or not they had bacterial infection.

Viral infection	Bacterial infection		
	Yes	No	Total
Yes	A	B	N_1
No	C	D	N_2
Total	M_1	M_2	T

NOTE. In this table the numbers of patients in each category are represented by the letters A, B, C, D, M_1, M_2, N_1, N_2, and T, which are used in the accompanying text.

The Mantel-Haenszel mean E (A) and variance V (A) for each table are computed in the following manner with use of the parameters defined in table 1.

$$E(A) = M_1N_1/T \text{ and}$$
$$V(A) = N_1N_2M_1M_2/[T^2(T-1)].$$

Table 2. Mantel-Byar computations for test of association between concurrent infections with influenza virus and *Streptococcus pneumoniae (S. pneu.)*.

Day of influenza infection	No. with influenza			No. without influenza							
	With *S. pneu.* (A)*	Without *S. pneu.* (B)	Total (N_1)	With *S. pneu.* (C)	Without *S. pneu.* (D)	Total (N_2)	A+C (M_1)	B+D (M_2)	T	E(A)	V(A)
395	0	1	1	3	43	46	3	44	47	0.0638	0.0598
446	0	3	3	8	59	67	8	62	70	0.3429	0.2949
521	0	1	1	7	76	83	7	77	84	0.0833	0.0764
816	0	1	1	8	35	43	8	36	44	0.1818	0.1488
823	1	0	1	2	11	13	3	11	14	0.2143	0.1684
837	1	1	2	3	36	39	4	37	41	0.1951	0.1717
890	0	1	1	11	60	71	11	61	72	0.1528	0.1294
1,181	0	1	1	11	50	61	11	51	62	0.1774	0.1459
1,212	0	1	1	1	15	16	1	16	17	0.0588	0.0554
1,233	1	0	1	8	54	62	9	54	63	0.1429	0.1224
1,503	0	1	1	7	44	51	7	45	52	0.1346	0.1165
1,518	2	0	2	1	23	24	3	23	26	0.2308	0.1959
1,572	0	1	1	11	49	60	11	50	61	0.1803	0.1478
1,914	0	1	1	3	16	19	3	17	20	0.1500	1.1275
1,918	1	0	1	1	47	48	2	47	49	0.0408	0.0392
2,647	0	1	1	8	54	62	8	55	63	0.1270	0.1108
Total	6*									2.4766*	2.1108

NOTE. Groups of patients indicated by letters are described in table 1. E = mean; V = variance.

*Number observed/number expected = $\Sigma A/E(\Sigma A) = 2.42$; χ^2 (1 df) = $(6-2.4766-0.5)^2/2.1108 = 4.33$; $P = 0.037$.

The ratio of the observed to expected (O/E) number of cases with both bacterial and viral infection is given by the equation $O/E = \Sigma A/\Sigma E$ (A) to summarize all tables.

Table 2 summarizes our analysis of the association between influenza virus infection and concurrent (within seven days) isolations of *S. pneumoniae*. To explain the construction of table 2, we must consider the events recorded for day 395 of the study. One patient was diagnosed on that day as having influenza virus infection ($N_1 = 1$); no influenza virus-infected patients had concurrent infection with *S. pneumoniae* ($A = 0$); and one influenza virus-infected patient had a culture negative for *S. pneumoniae* ($B = 1$). There were 46 control patients found to be free of influenza virus ($N_2 = 46$), three of whom had pneumococci ($C = 3$) and 43 of whom did not ($D = 43$).

This computation was made for each day when influenza virus infection was documented, and a total of six influenza virus infections ($\Sigma E = 6$) were found to be associated with isolation of pneumococci within the subsequent seven days (number observed, 6). The expected (E) number of concurrent influenza virus-pneumococcal infections, ΣE (A), was 2.4766, giving an O/E ratio of 2.42 (figure 1). The χ^2 value for a significant viral-bacterial interaction was 4.33 ($P = 0.037$) as calculated according to the method of Mantel and Byar [23].

Since many patients received antimicrobial therapy coincident with their virus-induced exacerbations, the possibility was considered that some bacterial infections may have been suppressed, thus masking a true association between viral and bacterial infections. To compensate for this effect, in the subsequent analysis, those individuals who were receiving antibiotic therapy during or 14 days prior to the day on which a bacterial culture was taken, and whose cultures were negative for the bacteria under study, were eliminated from analysis during that time period.

In the analyses described above, data from the 30 control subjects were pooled with data from the 120 patients with COPD. Separate analyses of the data from the 120 patients with COPD were performed and did not indicate an appreciable difference between the COPD group and the group as a whole.

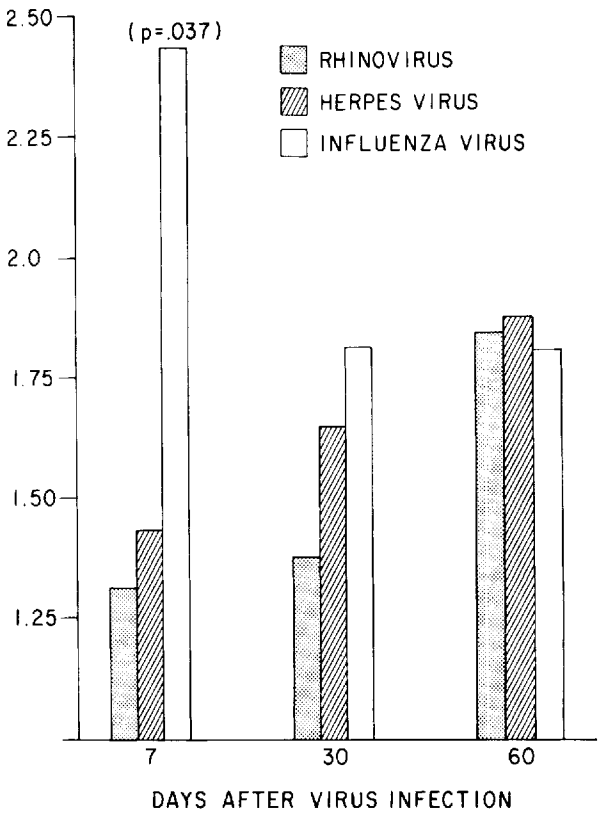

Figure 1. Ratios of number observed to number expected (ordinate), calculated by the procedure of Mantel and Byar [23], for the isolation of *Streptococcus pneumoniae* from sputum specimens and/or throat swabs within seven, 30, and 60 days after infection with rhinovirus, herpes simplex virus, and influenza virus. Significance was calculated by the χ^2 technique, and P values of <0.06 are noted.

Results

A total of 2,632 throat swabs and 1,533 sputum specimens obtained from the 150 patients on 2,708 occasions were cultured for detection of the presence of bacteria. In the subsequent analysis, an individual was considered positive for the bacteria in question when either the throat or the sputum culture was positive. Overall, the percentage of cultures positive for *S. pneumoniae* was 9.2%; for *H. influenzae*, 12.8%; and for *H. parainfluenzae*, 41.9% (table 3).

Viral infections were documented on 273 occasions either by culture or by serologic techniques. To permit analysis of the frequency of simultaneous bacterial and viral infections, we had to assign an exact date for the occurrence of each viral infection. When the viral infection was document-

Table 3. Numbers and percentages of persons with viral and mycoplasmal infections who had simultaneous bacterial infection.

Virus or mycoplasma	No. cultured for bacteria*	No. (%) with indicated bacteria		
		Streptococcus pneumoniae	*Haemophilus influenzae*	*Haemophilus parainfluenzae*
Herpes simplex virus	47	6 (12.8)	10 (21.3)	23 (49)
Rhinovirus	46	4 (8.5)	5 (10.6)	20 (43)
Influenza viruses (types A and B)	37	5 (13.5)	4 (10.8)	11 (30)
Parainfluenza viruses (types 1, 2, and 3)	16	1 (6.3)	2 (12.5)	3 (19)
Coronaviruses (OC 43 and 229E)	11	0	0	0
Respiratory syncytial virus	4	1 (25)	0	2 (50)
Adenovirus	3	0	0	1 (33)
Mycoplasma pneumoniae	4	1 (25)	2 (50)	1 (25)
Total	168	18 (10.7)	23 (13.7)	61 (36)
Percentage of total bacterial cultures positive		(9.2)	(12.8)	(41.9)

*Bacterial culture of throat and/or sputum taken at time of virus isolation (herpesvirus and rhinovirus) or at time of illness associated with antibody rise.

ed by isolation of the virus in culture (herpesvirus and rhinovirus), the date of viral culture was used. In the case of all other viral infections, demonstration of a fourfold or greater rise in titer of antibody in consecutive sera was required for diagnosis of infection. When an acute respiratory illness occurred during the same time period as the antibody rise, the date of the illness was also designated as the date of viral infection. Viral infections that were not associated with illness were not included in the analysis, leaving a total of 189 documented viral or mycoplasmal infections. To permit calculation of the rates of dual viral-bacterial infections, we included in the denominator only those viral infections that were simultaneously cultured for the presence of bacteria (table 3). Thus, a total of 168 viral infections remained available for this analysis.

A total of 102 viral infections were associated with the simultaneous isolation of *S. pneumoniae, H. influenzae,* or *H. parainfluenzae* from throat or sputum cultures (table 3). The percentages of viral infections associated with simultaneous isolations of *S. pneumoniae* (10.7%) and *H. influenzae* (13.7%) were somewhat greater than the percentages of total cultures positive for these bacteria (9.2% and 12.8%, respectively). The greatest associations of specific viral infections with isolations of these bacteria were between herpesvirus infections and isolations of *S. pneumoniae* (12.8%) and *H. influenzae* (21.3%), and between influenza virus infections and isolations of *S. pneumoniae* (13.5%). For all of the other virus-bacteria combinations studied, the percentages of dual viral-bacterial infections were similar to, or less than, the overall rates of isolation of these bacteria, or the numbers involved in the calculations of percentages were too small to be of significance.

The possibility that clustering of viral infections and the use of antibiotics might obscure a more significant association between viral infections and isolation of bacteria was considered in adapting the Mantel-Byar procedure for analysis of the data (see Materials and Methods). The association of rhinovirus, herpesvirus, and influenza virus infections and concurrent (within seven days) or subsequent (30 and 60 days) isolation of *S. pneumoniae, H. influenzae,* or *H. parainfluenzae* was calculated with the results expressed as a ratio of observed associations to expected associations (O/E). No important associations were detected between infections with the three viruses and the isolation of *H. parainfluenzae* in that all O/E ratios were less than 1.5. In contrast, there was an apparent association between infection with these three viruses and isolation of *S. pneumoniae* (figure 1) and *H. influenzae* (figure 2). The most significant viral-bacterial association was between influenza virus and concurrent isolation of *S. pneumoniae* ($O/E = 2.43$; $P = 0.037$). The association of herpesvirus and rhinovirus infections with isolations of *S. pneumoniae* was strongest in the 60-day analysis, in which

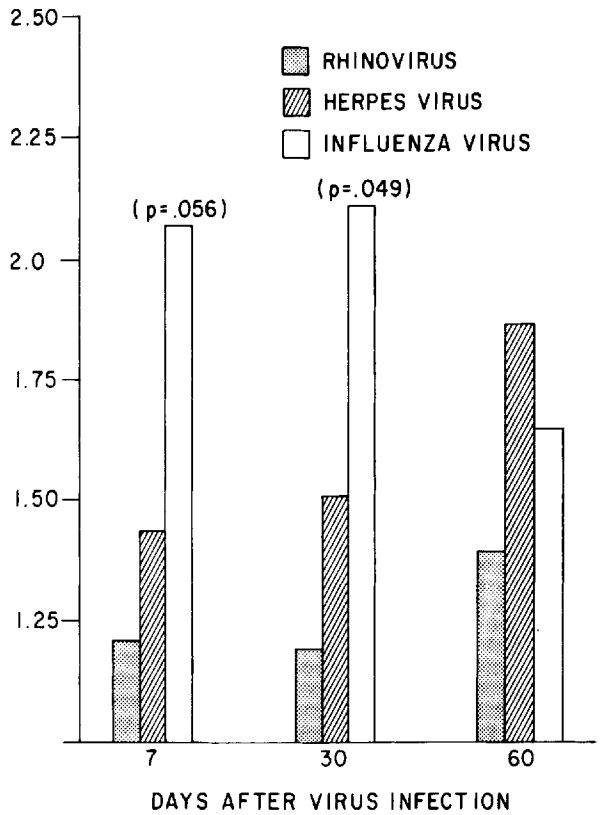

Figure 2. Ratios of number observed to number expected (ordinate), calculated by the procedure of Mantel and Byar [23], for the isolation of *Haemophilus influenzae* from sputum specimens and/or throat swabs within seven, 30, and 60 days after infection with rhinovirus, herpes simplex virus, and influenza virus. Significance was calculated by the χ^2 technique, and P values of <0.06 are noted.

both O/E ratios exceeded 1.75. Isolations of *H. influenzae* were also most significantly related to prior infection with influenza virus (figure 2). Positive, but not as strong, associations were detected between herpesvirus and rhinovirus infections and the subsequent isolation of *H. influenzae.*

Since many patients with COPD may be chronic carriers of each of the three bacteria studied, the Mantel-Byar procedure was modified by inclusion in the analysis of only those positive bacterial cultures that had been preceded by a culture negative for the bacteria in question. This approach eliminated those individuals who were chronically infected with *H. influenzae, H. parainfluenzae,* or *S. pneumoniae,* and it gave an indication of the rate of acquisition of new bacteria after viral infection. The results of this analysis indicated a greater association of herpesvirus with subsequent acquisition of *S. pneumoniae* (O/E for day 7, 1.76; day 30, 1.89; and day 60, 1.98) than was detected in the previous analysis in which all positive bacterial cultures were included (figures 1 and 2). For all the other viral-bacterial interactions, the associations of viral infections with subsequent acquisition of bacteria were less than those detected when all bacterial cultures were included.

The Mantel-Byar analysis was reversed to evaluate a possible association between bacterial infection and subsequent viral infections. Suggestive associations were observed between *S. pneumoniae* or *H. influenzae* infections and concurrent or subsequent isolation of herpes simplex virus (table 4).

To assess the invasiveness of *H. influenzae* in our study population, we tested 2,514 sera from the 150 patients for the presence of CF antibody with an antigen specific for the nonencapsulated *H. influenzae* group. Fourfold or greater rises in titer of antibody to *H. influenzae* were detected on 76 occasions in 53 patients.

Fourteen (18%) of the 76 rises in antibody to *H. influenzae* were associated with a viral or mycoplasmal infection during the preceding 60 days, and 20 (26%) were associated with such infections during the preceding 120 days (table 5).

Table 4. Ratios of number of observed to number of expected (O/E) herpesvirus infections subsequent to bacterial infections.

Bacteria	O/E on indicated day		
	Day 7	Day 30	Day 60
Streptococcus pneumoniae	7/4.81 = 1.46*	8/5.25 = 1.52	8/5.49 = 1.46
Haemophilus influenzae	11/8.63 = 1.28	12/8.13 = 1.48	14/8.80 = 1.60
Haemophilus parainfluenzae	30/26.3 = 1.14	35/29.3 = 1.20	37/30.3 = 1.22

*Ratios are the actual number of bacteria-positive individuals who were also positive for herpesvirus ÷ the number of individuals expected to be positive for the bacteria under study.

Table 5. Number of viral or *Mycoplasma pneumoniae* infections followed by fourfold or greater rises in the titer of CF antibody to *Haemophilus influenzae*.

Virus or mycoplasma	No. of infections	*H. influenzae* antibody rises	
		Day 60	Day 120
Herpes simplex virus	58	3	4
Rhinovirus	55	1	2
Influenza viruses (types A and B)	53	3	5
Parainfluenza viruses (types 1, 2, and 3)	40	3	3
Coronaviruses (OC 43 and 229E)	27	0	1
Respiratory syncytial virus	16	1	3
Adenovirus	14	0	0
M. pneumoniae	10	3	3
Total	273	14	20
No. expected*		8.25	8.34
No. observed/no. expected		1.70	2.40
P value		0.25	0.08

*Based on an analysis by the method of Mantel and Byar [23].

The Mantel-Byar method was also used to analyze the association of viral or mycoplasmal infections with subsequent rises in titer of antibody to *H. influenzae*; the O/E ratio at the 60-day interval was 1.70 ($P = 0.25$), and at the 120-day interval the ratio was 2.40 ($P = 0.08$). Influenza virus infection was most commonly associated with rises in antibody to *H. influenzae*, an observation that is consistent with the previously described association between infections with this virus and increased isolations of *H. influenzae* from the respiratory tract.

Discussion

Long-term studies of the role of infection in the pathogenesis of a chronic disease such as COPD are beset with a number of problems related to the collection and assessment of data. The initial problem of inhomogeneity of the study population was approached by a requirement that all patients with COPD have a clinical history compatible with chronic bronchitis or emphysema and evidence of obstructive airways disease as determined by testing of pulmonary function. Nevertheless, our COPD population contained patients with both mild and severe disease associated with a variety of primary diagnoses that included emphysema with α_1-antitrypsin deficiency, chronic asthma, and "nonspecific bronchitis" with a typical history of heavy smoking. When it became apparent that separate analysis of each subgroup of patients would partition our data to such a degree that it would be of little value, we elected to assess the entire study population en bloc. The 30 control patients were also included in the final analysis since their exclusion had no significant effect on our major findings. However, the possibility remains that viral-bacterial interactions may occur with different frequency in different subgroups of a population with COPD.

Short-term longitudinal studies of the relation of infectious agents to disease may be biased by variability in the incidence of infections with the seasons and from year to year. The fact that our period of observation extended over seven years tended to minimize the importance of this variability, and a major advantage of the Mantel-Byar analysis was that it permitted comparison of virus-positive patients with virus-negative controls studied during the same time period.

Many of our study patients were receiving antimicrobial therapy when bacterial cultures were taken. In this population, such therapy reduced the incidence of pneumococcal isolations by nearly 50% but had little effect on the rate of isolation of *H. influenzae* or *H. parainfluenzae* [24]. The potential problem of false-negative bacterial cultures in patients receiving antimicrobial agents was handled in the Mantel-Byar analysis by the discarding of such negative cultures from the analysis. Elimination of these negative cultures from the analysis did not alter the type of viral-bacterial association observed, i.e., O/E viral-bacterial ratios of >1.5 were detected on 11 occasions when negative cultures from patients receiving antibiotics were eliminated and on nine occasions when the cultures were kept in the analysis. Elimination of such negative cultures did have the effect of increasing the significance of some associations. For example, the greatest change occurred for the seven-day, influenza virus-*S. pneumoniae* analysis. The O/E ratio was 6/2.47 ($P = 0.037$) when negative cultures were eliminated and was 6/3.70 ($P = 0.31$) when all of the cultures were included.

As Glasgow [25] has pointed out, differentia-

tion between acquisition, colonization, invasion, and disease due to bacteria and viruses presents an important problem in clinical studies of viral-bacterial interactions. The Mantel-Byar analysis permitted us to study the question of acquisition vs. chronic colonization with bacteria and indicated that the strongest association was between viral infection and bacterial colonization. On the other hand, our analysis of the relation of viral infection to subsequent rises in titer of antibody to *H. influenzae* did suggest that viral infection may lead to increased invasiveness of *H. influenzae*.

A limitation of the Mantel-Byar method is the assumption that past history is of no significance to the current status of the patient [23]. Our consideration of prior antibiotic usage and prior colonization of the respiratory tract with the bacteria in question represents an attempt to account for those historical factors most likely to influence the results. Nevertheless, we were unable to account for other historical factors, especially previous infections, which may have occurred prior to the observation period. This situation is similar to that in any study, i.e., the results are conditional, depending on the lack of importance of any unknown factors or variables that cannot be observed. The fact that this study lasted for seven years should mitigate the effects of previous infections, and the movement of patients back and forth between study and control groups should reduce the effect of any bias based on unique past histories.

With these problems of collection and interpretation of data in mind, it was possible for us to identify several instances in which viral infections were associated with an increased incidence of concurrent or subsequent colonization with bacterial pathogens. The most significant associations by our analysis were between influenza virus infections and isolations of *S. pneumoniae* or *H. influenzae*. Considerable data from studies in laboratory animals and populations of normal adults indicate that influenza virus infections may predispose to bacterial infection and disease. Convincing evidence linking influenza viruses and *H. influenzae* was found in Shope's studies of synergy between these two agents in swine [26] and in studies with mice conducted by Sellers et al. [3]. Pfeiffer's reports of the original isolation of *H. influenzae* related this organism to epidemics of clinically significant influenza, but studies of subsequent epidemics suggested that *H. influenzae* was only an occasional secondary bacterial invader [1].

In the present study, *H. influenzae* was isolated more than twice as often as expected after influenza virus infection, and the invasiveness of *H. influenzae*, as judged by rises in antibody titer, was also increased after viral infection. Despite these findings, in no instance were we able to diagnose a serious *H. influenzae* infection such as pneumonia or empyema after a viral infection, possibly because in chronic bronchitis these organisms are most often of the nonencapsulated variety and, thus, probably are inherently less pathogenic than the encapsulated forms seen in children and normal adults [9].

Animal studies have also linked influenza virus infection with increased susceptibility to pneumococcal disease [2, 3, 34]. Finland [4] has summarized the clinical data relating these two organisms; he pointed out that in one epidemic 50% of patients with pneumococcal pneumonia had evidence of recent influenza virus infection. As was the case with *H. influenzae*, we were able to detect a significant association between influenza virus infection and colonization with *S. pneumoniae* but were not able to identify serious pneumococcal disease after influenza virus infection.

Although rhinovirus infections were associated more often with isolations of *S. pneumoniae* and *H. influenzae* than would be expected, the associations were not as strong as those seen after influenza. A relation between rhinovirus infection and bacterial infections of the respiratory tract was first suggested by Cherry et al. [6], who reported an increased rate of isolation of *S. pneumoniae* and *H. influenzae* from 11 children hospitalized with rhinovirus infections. One explanation for this association is suggested by the family studies conducted by Gwaltney et al. [15]. They noted that persons with colds were more effective transmitters of pneumococci than those without colds, and they documented simultaneous transmission of this organism and a rhinovirus by a child to other family members. A second explanation for the association between *S. pneumoniae* and common cold viruses is sug-

gested by the studies of Webster and Clow [27], who noted that the numbers and distribution of *S. pneumoniae* in the upper respiratory tract of chronic carriers increased coincident with the onset of colds. They hypothesized that respiratory viral infections may alter the milieu of the upper respiratory tract in a manner that favored increased growth of *S. pneumoniae*.

In contrast, Foy et al. [28] failed to detect an association between viral infections of the respiratory tract and isolations of *S. pneumoniae*. In a study of pneumococcal isolations from patients with pneumonia, they observed more viral infections in patients who did not harbor pneumococci (36%) than in those carrying pneumococci (26%). Possible explanations for this divergent observation include the broad age range of the patients studied and the failure to analyze the possible effects of antimicrobial therapy on the carriage of *S. pneumoniae*.

An association between viral infections and fourfold or greater rises in titer of antibody to *H. influenzae* was detected when all of the viral and mycoplasmal infections were considered as a group. To our knowledge, a relation between viral infections of the respiratory tract and changes in titer of antibody to *H. influenzae* has not been reported; however, several authors have reported such serologic responses in patients with chronic bronchitis following acute exacerbations, most of which were probably of viral origin [29–31].

Our attempts to relate herpesvirus infection to bacterial infections suggested an association between isolation of this virus and prior (within 30 days), concurrent (seven days), and subsequent infections with both *S. pneumoniae* and *H. influenzae*. In no instances, however, were the associations statistically significant. The long-held clinical impression that pneumococcal pneumonia may activate latent herpesvirus infection has recently been confirmed clinically by Fekety et al. [7] and in the mouse by Stevens et al. [32]. As Warren et al. [33] pointed out several years ago, however, fever itself is an important cause of activation of latent herpesvirus infection, and the relative roles of the organism (*S. pneumoniae*) vs. changes in the host environment (fever) remain to be elucidated.

Results of cultures for *H. parainfluenzae* were included in our analysis for purposes of comparison. Although most authors have chosen not to separate *H. parainfluenzae* from nonencapsulated *H. influenzae* [9, 13], we elected to test each isolate for both X and V factor requirements. This separation indicated that the nonencapsulated *H. influenzae* could be related to acute illness and severity of disease in patients with COPD, whereas *H. parainfluenzae* was of no pathogenic significance [34]. In the present report, isolations of *H. parainfluenzae* were in no instance suggestively related to concurrent or prior viral infections. We believe that this finding adds some weight to the significance of the association of viral infections with *S. pneumoniae* and *H. influenzae* that were described.

Our observations indicate that viral infections in patients with COPD are associated with increased rates of isolation of *S. pneumoniae* and *H. influenzae*, and that invasion of the latter organism, as judged by seroconversion, may also be a sequela of viral infection. This study supports the need for evaluation of the effectiveness of respiratory virus vaccines in patients with COPD and provides justification for further studies of the prophylactic and therapeutic value of antimicrobic agents in these patients.

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